Modulation of Lens Cell Adhesion Molecules by Particles

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Abstract

Cell adhesion molecules (CAMs) are proteins which anchor cells to each other and to the extracellular matrix (ECM), but whose functions also include signal transduction, differentiation, and apoptosis. We are testing a hypothesis that particle radiations modulate CAM expression and this contributes to radiation-induced lens opacification. We observed dose-dependent changes in the expression of β1-integrin and ICAM-1 in exponentially-growing and confluent cells of a differentiating human lens epithelial cell model after exposure to particle beams. Human lens epithelial (HLE) cells, less than 10 passages after their initial culture from fetal tissue, were grown on bovine corneal endothelial cell-derived ECM in medium containing 15% fetal bovine serum and supplemented with 5 ng/ml basic fibroblast growth factor (FGF-2). Multiple cell populations at three different stages of differentiation were prepared for experiment: cells in exponential growth, and cells at 5 and 10 days post-confluence. The differentiation status of cells was characterized morphologically by digital image analysis, and biochemically by Western blotting using lens epithelial and fiber cell-specific markers. Cultures were irradiated with single doses (4, 8 or 12 Gy) of 55 MeV protons and, along with unirradiated control samples, were fixed using –20^{o}C methanol at 6 hours after exposure. Replicate experiments and similar experiments with helium ions are in

progress. The intracellular localization of β1-integrin and ICAM-1 was detected by immunofluorescence using monoclonal antibodies specific for each CAM. Cells known to express each CAM were also processed as positive controls. Both exponentially-growing and confluent, differentiating cells demonstrated a dramatic proton-dose-dependent modulation (upregulation for exponential cells, downregulation for confluent cells) and a change in the intracellular distribution of the β1-integrin, compared to unirradiated controls. In contrast, there was a dose-dependent increase in ICAM-1 immunofluorescence in confluent, but not exponentially-growing cells. These results suggest that proton irradiation downregulates β1-integrin and upregulates ICAM-1, potentially contributing to cell death or to aberrant differentiation via modulation of anchorage and/or signal transduction functions. Quantification of the expression levels of the CAMs by Western analysis is in progress.

Key Words

β1-integrin; ICAM-1; protons; human lens epithelium.

Introduction

Cell Adhesion Molecules (CAMs) are important to the regulation of cell motility, division, differentiation and apoptosis (1,2). CAMs link cells to the extracellular matrix and mediate mechanical and chemical signals from them. These signals regulate kinases,

growth factor receptors and ion channels, and control the organization of the cytoskeleton (3). Radiation effects on CAMs have been reported elsewhere (4,5).

The study of mechanisms underlying proton radiation-induced cataractogenesis is important for determining risks of development of late tissue effects during space travel. Basic Fibroblast Growth Factor (FGF-2) has an essential role in the proliferation, migration and differentiation of human lens epithelial cells. We have previously reported rapid up-regulation of FGF-2 transcription in response to proton exposure (6).

We are investigating radiation-induced changes in two CAMs, β_1-integrin and ICAM-1, in an *in vitro* primary human lens epithelial (HLE) model system (7).

Materials and Methods

Cells

Normal Human Lens Epithelial (HLE) prenatal cells (passage 4-10) were grown in Dulbecco's MEM (low glucose) with 15% FBS, plus antibiotics on extracellular matrix (ECM) derived from bovine corneal endothelial cells and supplemented with 5 ng/ml of FGF-2 extracted from bovine pituitary. The cultures were maintained at 37^{o}C in a humidified atmosphere of 10% CO_2. Cells were set up in 60 or 100 mm ECM dishes at 2 x 10^4 cells/cm^2 for exponential (E), 1 week (1C) or 2 week (2C) confluent cultures, 3, 12, or 20 days respectively before an experiment. Cells were fed every other day with growth media plus FGF-2.

Neutralizing Antibody Treatments

Neutralizing anti-FGF-2 polyclonal antibody (AB-233NA R&D Systems, Minneapolis, MN) was added to cultures at 30 μg/ml in 1% FBS growth media minus FGF. Cultures were rinsed 2X in 1% FBS growth media, and NAb was added for 1 hour before radiation. The volume of media was reduced during the radiation (due to being irradiated vertically), and was replaced immediately after radiation and the cultures were incubated for 6 hours at 37^{o}C.

Radiation sources

55 MeV/amu Proton ion beam exposures were made at the 88" cyclotron at LBNL. The dose-averaged linear energy transfer (LET) of the Proton beam is calculated to be 1.18

keV/μm. Parallel plate ionization chambers were used for dose measurements. The dose rate used was between 2 - 4 Gy/min.

Immunofluorescence

Cells grown on ECM were rinsed twice with Ca^{2+} and Mg^{2+} free PBS, fixed with -20^{o}C 100% Methanol for 5 minutes and air dried. Cells were rehydrated and permeabilized with PBS with 0.1% NP40, then blocked with 10% goat serum, for 20 minutes at R.T. Primary antibodies specific for ICAM-1 and β_1-Integrin (Oncogene, Cambridge, MA) were applied at 20 μg/ml in 2% BSA for 1 hour. The secondary antibody used was Alexa Fluor™ 594 goat anti-mouse IgG (H+L) conjugate (Molecular Probes, Eugene, OR), diluted 1:100 in PBS. Cells were counterstained using 0.25 μg/ml DAPI, and mounted with vectashield. The samples were examined at 40x magnification using an Olympus microscope, and photographed using a Spot™ digital camera and software. Quantitation of the fluorescence was accomplished using a method developed by S.J. Lockett using SCIL-Image™ software.

Results and Conclusions

- The constitutive expression of adhesion molecules is dependent on the differentiation status of the lens cells.
- The cellular distribution of β_1-integrin expression changes in the transition from lens epithelial to fiber cell. The β_1-integrin moves from a focal adhesion point to a more diffuse distribution.

- The differentiation of human lens epithelial cells into fiber cells *in vitro* correlates with increased expression of β_1-integrin and little expression of ICAM-1.
- Radiation induces changes in the immunoreactivity of adhesion molecules.
- Exponential cells show a dose-dependent increase in β_1-integrin expression up to 8 Gy with changes in intracellular localization, but a decrease in immunoreactivity in the differentiated 1C and 2C cells at 6 hrs after exposure to 55 MeV/amu protons.
- Only a small increase in ICAM-1 expression is observed in 2C cells after exposure to high doses of proton radiation.
- FGF-2 protein plays a role in modulating the levels of CAM expression and its radiation responsiveness.
- Treatment with NAb to FGF-2 alone for 7 hours dramatically increases the expression of β_1-integrin in E cells, but decreases expression in C cells. ICAM-1 expression shows no change in E cells and little change in C cells with treatment with NAb to FGF-2.
- Treatment with NAb to FGF-2 reduces the dose-dependent decrease in β_1-integrin and gives a more dramatic increase in ICAM-1 observed 6 hours after radiation exposure.
- Studies of the time course of radiation-induced changes in CAMs at lower radiation doses are in progress.

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